Complete Regenerative Peripheral Nerve Interfaces, Fatigue and Recovery

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Abstract — **Modern technology has taken great strides to restore motion to amputees with prostheses. A key limitation in many cases is lack of a reliable controlling interface to the prosthetic devices. To address this issue, our lab has developed the Regenerative Peripheral Nerve Interface (RPNI). RPNIs transduce signals between residual peripheral nerves, muscle grafts, and prosthetic devices. Prior to this study, RPNI's signal production was primarily evaluated during single evoked maximal action potential. The purpose of this study was to characterize RPNI function during and after repeated submaximal use. RPNIs (n=5) were constructed in a rat model by transferring the EDL muscle from the lower hind limb to the hip region and implanting the transected peroneal nerve into the muscle. Control EDL muscles (n=8) were left in the native location. The muscles were evaluated at least five months post-operatively in terms of maximum evoked compound muscle action potentials, force production, force production during repeated use, and post-fatigue force production. There was a strong correlation between maximum compound muscle action potential amplitude and maximum contractile force (r=0.83, p < 0.01); thus, force was an indication of signaling. RPNI and Control muscles both fatigued as exponential regressions. Percent post fatigue force production did not differ significantly between the groups, with Controls recovering to 85% of initial maximum force and RPNIs recovering to 60%. RPNIs produce and recover signals in the same relative manner as Controls indicating RPNIs are prime candidates as controlling interfaces for myoelectric prosthetic devices.**

Keywords—fatigue; peripheral nerve; CMAP; skeletal muscle; regeneration; re-innervation.

I. INTRODUCTION

Current myoelectric prosthetic devices are able to accurately mimic the natural movements a human performs with their native upper extremity. Unfortunately, amputees do not find using these modern myoelectric prostheses worth the time it takes to learn to control them. Current interfaces use skin surface electrodes as the interface between underlying muscles and prostheses electronics. The amputee needs to retrain himself to activate select muscles underlying the surface electrodes in order to produce the desired movement of the prosthesis. For advanced prostheses to be a feasible solution for an amputees, there must be an intuitive link between the amputee's volition and the prosthesis. Many systems such as

targeted muscle reinnervation (TMR) and direct implantation of multi-electrodes into nerves are being perfected. However, controlling interface units must be reliable, convenient, long lasting and function independently. The Regenerative Peripheral Nerve Interface (RPNI) is a newer interface unit that embodies more of the desirable characteristics than other methods and, most importantly, provides intuitive control [1-5].

An RPNI unit (Fig 1) is made of a muscle graft that has been neurotized by transected peripheral nerve fibers from the residual limb. An electrode is placed on the graft to record the signals being produced by the nerve and subsequent activation of muscle fibers. The large muscle graft depolarization during activation amplifies signals from the nerve. The muscle tissue also prevents damage that might occur from placing an electrode directly on or into the nerve. Further, with RPNI units being implanted permanently in the body, the method addresses the problem of electrode slippage experienced by surface electrode methods [6].
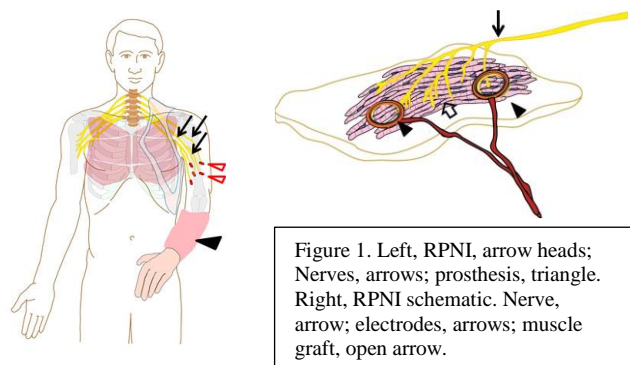

Figure 1. Left, RPNI, arrow heads; Nerves, arrows; prosthesis, triangle. Right, RPNI schematic. Nerve, arrow; electrodes, arrows; muscle graft, open arrow.

For this model to be effective, the endurance characteristics of the muscle must not be lost in the RPNI. A theoretical user of an RPNI needs it to work repeatedly to perform simple, daily tasks without fatigue or failure. For instance, if a person was using their prosthesis to type on a keyboard, they need to know it will work the whole time, and continue to work later in the day.

Thus, our purposes were to (1) Determine how the RPNI muscle fatigues during submaximal repeated use and (2)

Compare post-fatigue recovery capabilities of RPNI muscles to normal muscles.

II. METHODS

Overview

All procedures were performed in accordance to the ethical guidelines of the University of Michigan's University Committee on Use and Care of Animals [7]. Rats (Harlan, F344) were randomly assigned to one of three experimental groups. Groups were titled RPNI, Control, and Denervated. At time points ranging from five months to thirteen months post-operatively, rats were evaluated and sacrificed. The evaluation procedure and outcome measurements are outlined in the Endpoint Physiological Testing section. Data were analyzed using IBM SPSS 21.

Initial Surgery

Each RPNI group rat (n=5) had the extensor digitorum longus (EDL) muscle freely transferred from its native location to the upper thigh as in [8]. During muscle transfer, the native neurovascular pedicle was divided. The transferred muscle was neurotized by the transected peroneal nerve. The cut tendons were secured to the femur, and an epimysial recording electrode – either fine wire or stainless steel pad – was sutured to the muscle. The electrode cable was run subcutaneously along the length of the leg, lateral to the spine and secured in a headcap on the skull of the rat. The entire RPNI construct was wrapped in porcine small intestinal submucosa (SIS). The Control group (n=8) had one of the aforementioned electrodes sutured to the intact EDL muscle that remained in the native anatomical location. Tendons were severed and repaired. The Denervated group (n=3) had identical procedures as the RPNI group except the residual peroneal nerve was not implanted into the EDL muscle. All skin incisions were closed in layers and allowed to convalesce for at least five months.

Endpoint Physiological Testing

At endpoint testing, each rat was weighed, analgesia was administered (carprophen) and anesthesia administered (isofluorane or pentobarbital) for a deep plane of unconsciousness. The RPNI, Control, or Denervated muscle construct was dissected through a skin incision with subsequent muscle splitting exposure. The electrode and cable implanted during the initial surgery were removed. The peroneal nerve was subsequently also dissected free from underlying fascia. A bipolar stimulating electrode was placed under the peroneal nerve. Platinum needle electrodes were placed in the muscle belly (sensing electrode), the distal tendon of the muscle (reference electrode), and between toes four and five (ground electrode). The following CMAP and Force testing methods were based off of [9].

CMAP Testing

Compound muscle action potential values (CMAP) were measured using a Viasys™ System. Single 0.1 ms. stimulation pulses beginning at 0.03 mA and increasing by 0.03 mA were delivered until a muscle electromyograhic (EMG) twitch response was initially recorded. The lowest stimulation current to induce a recorded visible muscle response was defined as the rheobase (mA). Stimulation was then incrementally
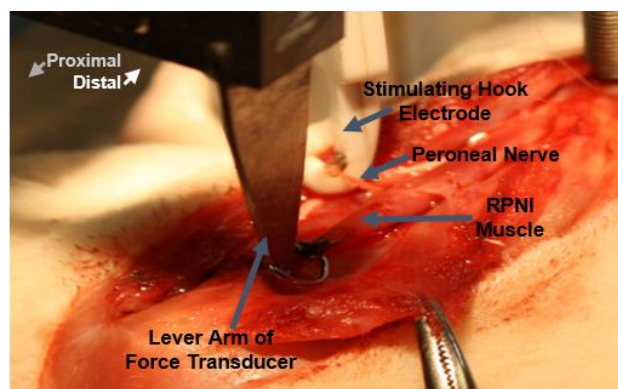

Fig. 1. Close up of rat in situ force measuring setup with an RPNI (muscle transferred to the upper thigh). The neurotizing peroneal nerve is stimulated by the hook electrode, evoking a contraction of the RPNI muscle, which pulls on the lever arm of the force transducer.

increased until the amplitude of the response no longer increased. The amplitude of the EMG response was defined as the maximum CMAP amplitude (mV).

Force Testing

One EDL muscle tendon was detached on either the proximal (for RPNIs) or distal (for Controls) end to the spine. The cut tendon was then secured to a small, wire ring that was used for attachment to the force transducer. The rat was positioned on a heated testing platform. The kneecap was pinned and the foot secured to ensure isolation of the EDL muscle. The muscle was then attached to the lever arm of the force transducer (Aurora Scientific™), and a stimulation hook electrode was placed on the exposed peroneal nerve, between 0.5cm and 2cm away from the muscle (Fig. 1). The rats' body temperature was monitored throughout the force testing and kept near 37°C. The nerve and muscle were bathed with warm mineral oil throughout testing to keep tissues moist and insulated. The muscle was stimulated indirectly through the peroneal nerve with increasing current for single, 0.2ms pulses until a maximum twitch force value was obtained and the stimulation current was noted. The muscle was then lengthened incrementally by 0.1mm until the maximum twitch force value plateaued. The muscle length providing maximum twitch force was defined to be optimal length (L_o). The muscle was then stimulated at the same current for 300ms at 30Hz to tighten the muscle. A final twitch was administered to ensure optimal length after the 30Hz stimulation. Next, the muscle was subjected to tetanic stimulations of increasing frequency for 300ms with two minutes for rest between each contraction until the force value plateaued. That was defined as the maximum tetanic force value (mN). After finding the maximum tetanic force, a fatigue protocol was administered to the muscle.

Fatigue Protocol

The fatigue protocol consisted of 6 sets of 120 evoked muscle activations at 40Hz with a 1.7 second rest between each stimulation pulse. For the first three sets, stimulation was held for 300ms, while stimulation for the latter three sets was held for 600ms. Two minutes of rest were allowed between fatigue sets, except after the third and sixth sets, where the muscle was allowed five minutes rest before stimulation to induce

maximum tetanic force. The amount of force produced after the fatigue protocol divided by the initial maximum tetanic force was defined to be the percent of maximum force recovered.

Muscle Harvest, Sacrifice and Histology

After testing, the muscle, nerve and electrodes were harvested, weighed, and photographed. Rats were then humanely euthanized.. Fixed muscle tissues were sectioned and stained with haematoxylin and eosin (H&E) as well as acetylcholinesterase (AChE) to observe relative muscle health and neuromuscular junction formation.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 21 (IBM Corp. 2012. Armonk, NY: IBM Corp.). Independent t-tests assuming unequal variance were used to compare means

TABLE I. Summary data for dependent variables by experimental group.

	Experimental Groups		Significance
	Control	*RPNI*	
n =	8	5	-
Body Mass (g)	417±45	433±26	0.436
Muscle Mass (mg)	171±22	164±48	0.768
Max CMAP Amplitude (mV)	22±10.37	3.7±3.4*	0.003
Maximum Tetanic Force (mN)	2577±565	627±427*	0.000
Max Force Recovered (%)	85±24	60±23	0.139

Data are presented as means ± standard deviation. Comparisons were by independent t-tests with two-tailed p≤0.05 considered significant (*). Abbreviations: mg, milligrams; mN, milliNewtons; mV, milliVolts; CMAP, compound muscle action potential.

between Control and RPNI groups with two-tailed p≤0.05 considered significant. Denervated group data were not included in statistical analysis.

III. RESULTS

CMAP and Force Production

The groups did not differ by body mass or post-operative days. Many of the electrodes and cabling implanted during surgery were not functional at end point testing due to cable breakage or electrode migration off the RPNI or EDL muscle.

Descriptive data for the RPNI and Control groups are summarized in Table 1. Maximum CMAP amplitude and tetanic force production was significantly larger in the Control group than the RPNI group. A tight correlation (r=0.83, p<0.01) was found between max CMAP amplitude and maximum contractile force in both the Control and RPNI groups, consistent with previous literature [10]. Denervated group muscles did not produce any EMG signals or muscle force as expected. The Denervated group was included to verify recordings for EMG and force data were indeed isolated only for the RNPI biological construct and the EDL muscle.

Muscle Endurance and Recovery

Both Control and RPNI muscles were able to repetitively produce EMG signaling throughout the entire fatigue protocol and each group demonstrated a decrease in the signaling indicating muscle or neuromuscular interface fatigue. Both groups showed fatigue from submaximal activation that tightly fit exponential regressions with coefficients of determination, R^2, equal to 0.94 for Controls and 0.89 for RPNIs, indicating a plateauing effect of force production during repeated use (Fig. 2). Following the six sets of the fatigue protocol and a five minute rest, Control and RPNI muscles recovered a large percentage of their force producing capabilities. Control muscles recovered 85% of their initial maximum tetanic force, while RPNIs recovered 60%.
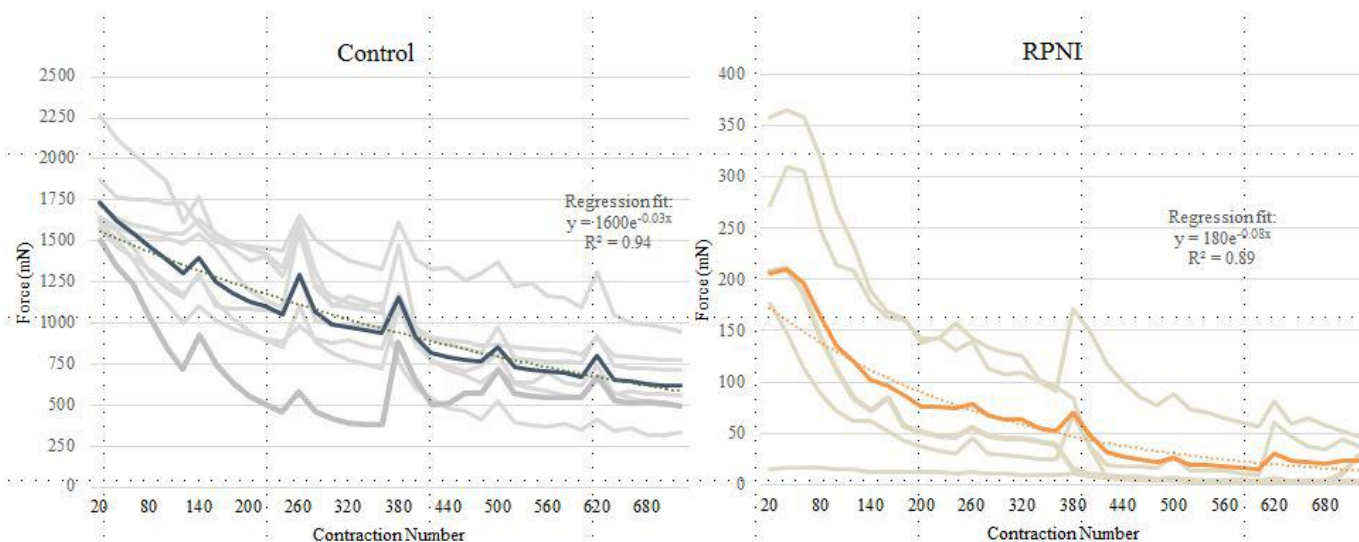

Fig. 2. Control group (left) and RPNI group (right) charts show the muscle force degradation during the 40 minute fatigue protocol. The light lines are traces of individual rats, while the bold lines are running averages. Both Control and RPNI and muscles tightly fit exponential regression models. Importantly, RPNI muscles continue to produce signals during prolonged, repetitive activation as do control muscles.
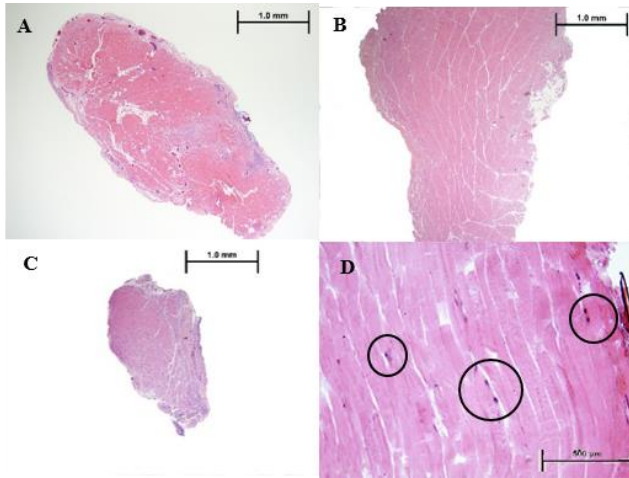
Fig. 3. A, B, and C show a cross section of EDL muscle in the RPNI, Control, and Denervated groups stained with H&E. Note the large, healthy muscle cells in both the RPNI and Control groups, compared to the small, degenerating muscle cells in the Denervated group. D shows a longitudinal section of an EDL RPNI muscle stained with AChE. This stain shows neuromuscular junctions as localized blue dots, circled above. Together, these pictures demonstrate a healthy RPNI muscle that has regenerated neuromuscular junctions.

Histology

Qualitative histological analysis confirmed regeneration of the RPNI muscles, as well as the reformation of neuromuscular junctions after neurotization by the transected peroneal nerve with nerve fiber sprouting and innervating regenerated muscle fibers over time (Fig. 3). Both RPNI and Control muscle sections stained with H&E showed large, healthy muscle cells compared to the small, degenerating muscle cells seen in the Denervated group. RPNI AChE stain confirmed formation of neuromuscular junctions.

IV. DISCUSSION

This study verified that RPNIs can transduce signals between residual peripheral nerves and prosthetic devices. RPNI signal production was evaluated by single evoked maximal action potentials and was also characterized during and after submaximal, fatiguing use.

Although CMAP and force values in the RPNI group were smaller than Controls, they were still robust by comparison to the absent signal found in the Denervated group. This is a very important result because the RPNI muscle had to regenerate most of the muscle fibers, the peroneal nerve needed to extend sprouts through the muscle tissue, and a mature blood supply needed to reform by diffusion, inosculation and re-vascularization after free muscle transfer.

The CMAP and force correlation further justifies using force methods to show potential signal degradation. Although other methods of evaluating the extent of muscular fatigue exist, such as studying the depletion of glycogen stores [11], that methodology requires ligating blood supply to the muscle

and is appropriate for this model. That RPNI muscles continued to contract throughout the fatigue protocol with a trajectory similar to Control muscles verifies that the RPNI muscle are healthy and are not injured during repetitive use even when an electrode has been implanted. The muscle retains normal fatigue characteristics after being transferred, implanted with and electrode, neurotized with transected nerve, wrapped with SIS, and allowed to recover. This is crucial to a potential user of an RPNI for prosthetic control – it is important that the signals being produced throughout an average day's work do not degrade to unusable levels and that after activity, the muscle is able to recover and produce signals again as it would naturally.

Though TMR is a similar approach to prosthetic device control it has several drawbacks that the RPNI resolves. We acknowledge that TMR is currently available to select patients. However, the nerves are less efficient in reinnervating muscles because of the existing innervation. Denervated muscles as in RPNI muscle grafts are more receptive to reinnervation. Also the electrodes for RPNI units are implanted with the muscle graft. No skin surface electrodes are used. Skin electrodes may shift as the body moves or sweats. The use of RPNI units should require less training and prosthesis movements will be more intuitive because the nerve fibers are captured distal to the brachial plexus, which makes the RPNI model more suitable for patients that retained part of their arm after amputation.

Some limitations of the study were that in some cases electrode materials elicited a biological response that resulted in electrode encapsulation. Further, only motor, and not sensory, function was studied here. With sensory nerve innervation, RPNIs also have the potential to restore sensory feedback to the user [12].

In summary, we were able to address the two main questions of this study: (1) how do RPNIs fatigue? and (2) how do they recover from fatigue? The fatigue and recovery results enable us to conclude that RPNI muscles tire as Control muscles do, and recover from fatigue as well as Control muscles in a rat model.

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